TRANSLATION 730 (T730)
MEDICAL ZOOLOGY DEPARTMENT
UNITED STATES NAVAL MEDICAL RESEARCH UNIT No. 3
CAIRO, EGYPT

TRANSLATION FROM RUSSIAN. SIDENKO, V. P., GREKOV, V. S., STEPANKOVSKAYA, L. D., POLYAKOV, E. M., SOLOMKO, R. M. & VOLYANSKAYA, E. A. (1973)*. Birds of southern Ukraine - reservoir of tickborne encephalitis. Sborn. Trud. Ekol. Virus., 1:140-144.

In accordance with the plan of coordinated study of arbovirus infection focus in south European USSR, in Black Sea regions of Ukrainian Republic, we made investigations to clarify tickborne encephalitis (TBE) infection in wild birds, participation of these birds in virus circulation, and formation of TBE natural foci in the Black Sea region, Ukraine.

MATERIAL AND METHODS

The material was collected in 4 bird migration and nesting areas: Stensov marshy-floodplain of the Danube River, Mologa locality of the Dniester River estuary, Bolshoi Sokoliny and Bolshoi Bokaisky islands, and Crimean foothills. Birds were shot and trapped (during banding period) throughout the entire year.

Bird sera were examined by the complement fixation (CF) test in Tokatsy microtitrator. In some cases, the bird heart suspension in borate solution (pH-7.0) was also examined by CF. TBE virus served for diagnostic tests.

Before virological tests, the brain, and parenchymal organs were removed from birds and placed in penicillin vials on dry ice.

Internal organ fractions of a few birds were used for preparing a suspension. These fractions were ground in a porcelain mortar adding 10-fold Hank's solution pH-0.7-7.6 or buffered physiological solution with 5-10% normal rabbit or calf serum and 100-200 streptomycin or penicillin units per 1 ml. The suspension was centrifuged at 2,000-2,500 rpm for 7-10 min. The supernate was used (0.02 ml) for infection of newborn white mice (NWM). Infected mice were observed for 21 days.

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The brain was removed from ill mice and used for further passages in the form of a 10% brain suspension.

Isolated agents were investigated in 3-4 passages. CF antigens to the studied viruses were made by the sucrose-acetone method (Clarke and Casals 1958). Standard hyperimmune rat and rabbit sera and immune ascitic fluids (IAF) to polygroup A, B, and TBE encephalitis viruses were used as specific serum (Gaidamovich et al. 1969).

RESULTS

A total of 2,010 birds of 53 species was serologically examined. We trapped 275 birds in the spring, 1,630 in summer, and 105 in fall.

Ninety-three bird brain samples and parenchymal organs of 339 birds of 22 species were virologically examined.

Of 2,010 bird blood samples, 56 contained CF antibodies to TBE virus (titers 1:8, 1:64, and higher), i.e. 2.7 $\pm$0.1%.

The maximum level of immunological responses was recorded in forest birds, such as sparrow and woodcock.

Table 1 show results from virological investigations of bird organs.

Isolated strains were apathogenic for guinea pigs and rabbits and their titers in tests on white mice were: 5.0 by intracerebral, 4.6 by intranasal, 3.8 by intravenous, and 1.8 lg LD_{50}/0.02 ml by intraperitoneal inoculations of strain 54, and 6.5 by intracerebral, 5.3 by intravenous, 3.2 by intranasal, and 2.0 lg LD_{50}/0.02 ml by intraperitoneal inoculation of strain 59.

The subcutaneous inoculation method was unsuccessful. The incubation period of infected mice fluctuated between 4 and 5 days. Experimental infection was characterized by disorders in coordination of movements and death of animals.

Chicken embryos (9-10 days old) proved also to be sensible to the viruses isolated. Strains 54 and 59 reproduced in the allantoic cavity and their titers were 2.5-4.0 lg. The highest mortality rate of embryos inoculated with a dose of 10 LD_{50} was observed on day 3-5 following infection.

For identification of strains, we used the CF test with standard hyperimmune sera (Table 2).

As seen in this table, the studied strains reacted with IAF of poly-B, IAF of standard hyperimmune serum against TBE, and also with homologous antisera. Consequently, CF cross reactions showed that strains 54 and 59 belong to TBE complex viruses.

In addition to virological investigations, we also observed infestation of birds with ticks and natural infection with viruses in parasites collected. We recorded that 6 bird species are hosts of ixodid tick larvae and nymphs: pheasant (Phasianus colchicus L.), blackbird (Turdus merula L.), tree pipit (Anthus trivialis L.), whitethroat (Sylvia communis Lath.), Calandra lark (Melanocorypha calandra L.), and yellow wagtail (Motacilla citreola Pall.). The highest tick numbers were recorded on birds (2nd 10 days of May) of Crimea. Ixodid ticks were absent on birds in winter.

Investigations of immature ticks for the presence of TBE virus gave negative results. One virus strain of group B (not belonging to TBE) was isolated from a pool of engorged larvae and nymphs collected from blackbirds. This strain is still being studied.

SUMMARY (Original in English)

In the blood of birds caught and shot on the territory of Ukrainian Prichernomorye, we detected complement-fixing antibodies to tick-borne encephalitis (TBE) virus. The vectors of TBE virus were both resident and migratory (rooks) birds.

LITERATURE

1. GAIDAMOVICH, S. Ya., L'VOVA, A. I., ABUKHOVA, V. R., et al. (1969). Vop. Virusol. (6):676. - 2. CLARKE, D. H. & CASALS, J. (1958). Amer. Jour. Trop. Med. Hyg., (7, 5):561.

Table 1. Results from virological investigation of birds in 1970-1972

Bird species	No. examined	No. bioassays	No.* strains isolated	No. of TBE virus strain
Gulls (Larus)	78	12	2	-
Coot	51	8	1	-
Sandwich tern	2	2	1	-
Little egret	16	4	1	-
Squacco heron	1	1	1	-
Garganey	1	1	1	54
Rook	102	31	7	-
Lark	7	5	2	-
Tree sparrow	23	3	2	59
Pheasant	3	3	1	-
Other species	41	23	-	-
Total	339	93	19	2

* The data on antigenic group B arboviruses are cited in other reports.

Table 2. Results from identification of strains 54 and 59 by the CF test

Serum \ Antigen	Strain 54	Strain 59	JE strain	TBE strain
IAF of poly -A	0	0	0	0
IAF of poly -B	20	40	20	40
IAF of TBE	40	40	0	40
Hyperimmune TBE	80	80	0	160
West Nile	0	0	20	0
Japanese encephalitis	0	0	80	0
Strain 54	40	40	0	0
Strain 59	20	40	0	40

Footnote: O - negative result; numbers show reciprocal sera titers with 2-4 antigen units.

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